



## CHAPTER V

### RESEARCH METHODOLOGY

#### RESEARCH DESIGN

Descriptive cross-sectional study is used in this study. It is the suitable design for prevalence survey. The factors that are associated with iron status of the subjects can be identified at the same time as the survey of prevalence. Although cross-sectional study is not a suitable design for identifying the associating factors because it can not identify clearly the sequence of the events . However , it may give some information as baseline data for further study.

Since iron deficiency anemia is a chronic disease which has a low mortality rate, therefore the prevalence rate can determine the size of the problem in the community.

The cross-sectional study has limitation in finding the factors causing the disease because it can not identify clearly the sequence of the events.

#### TARGET POPULATION

The target population for this study is the non pregnant women in reproductive age group, age between 15 and 45 years old, who live in poorly-developed villages of the Northeastern part of Thailand.



## SAMPLE POPULATION

The non pregnant women age between 15 and 45 years who live in the poorly developed villages of Amphoe Ban Fang, Khon Kaen Province and meet the eligibility criteria.

### Eligibility criteria

#### 1. Inclusion criteria

All women whose age are between 15 and 45 years old.

#### 2. Exclusion criteria

Subjects will be excluded from the study if one or more of the following condition/s exist/s :

- 1) pregnancy
- 2) Lactating
- 3) Chronic disease that need regular treatment at the hospital
- 4) Fever
- 5) Resent recovery from illness within 2 weeks.
- 6) Receiving iron supplement.
- 7) Menopause

## SAMPLE SIZE

1. The data in this study are proportional data.
2. The latest survey of prevalence of anemia at Amphoe Phon, Khon Kaen by Sirijerachai in 1988 showed prevalence of anemia among female reproductive age group 26.4 %. From the assumption that most of anemic

cases among this group of population caused by iron deficiency ,the estimate prevalence of anemia among the target population is about 25%

3. The acceptable error in this study is set at 5% . So " d " is = 0.05

4. The study is a descriptive study , it has one sample group ,so only type I error with confidential level of 95% is considered.

5. Cluster sampling using villages as the sampling unit was used in this study , consequently it will decrease the effective sample size calculated by the formular used in simple random sampling. To compensate for the reduction of the power to estimate the real prevalence , the sample size was multiply by the inflation factor , the design effect. The design effect depends on the variation of the cluster unit . Since it is no available data about the variation of the prevalence of anemia in each village ,therefore the design effect value shoud be select by estimation . The estimated subjects in each household was 2 cases , so the design effect of two is selected in this study.

5. The formula for calculation of sample size is :

$$n = \frac{Z_{\alpha}^2 * p (1-p) K}{d^2}$$

Where : n = sample size

p = estimate proportion of iron deficiency anemia  
in target population = 0.25

d = allowable error = 0.05

$\alpha$  = 0.05

$Z_{\alpha}$  = 1.96

K = design effect for cluster sampling = 2

Hence : Sample size = 576 cases.

In this study 629 cases were examined.

## STUDY SETTING

Amphoe (district ) Ban Fang is about 22 kilometers from Khon Kaen. It has 6 Thambol (subdistrict ) with 72 villages. According to the classification of villages by the Department of Community Development, 25 villages are poorly developed villages. The number of population of Amphur Banfang is about 40,000, half of them are females.

Banfang has area about 393 square kilometers . It has one "10 beds community hospital " with two doctors . Most of the primary health care provided by primary health care center . Each Thambol has it's own primary health care center. There are 6 primary health care center in the area of Amphoe Ban Fang.

Most of the population are farmers. The important products of this area are rice, sugar cane and corn. Since most of the population are poor, therefore migration to big city or other province for working is popular.

The Department of Community Development classified the villages as "developed"," moderatly developed" and "underdeveloped" , using 37 indexes as the indicator.The villages which have lower scored than standard more than 10 indexes were classified as underdeveloped villages. The indexes were income , migration rate , everage level of education , land holding , health service , health status of the population , sanitation , water supply , electricity , transportation ,etc.

There are 25 villages of Amphoe Ban Fang which were classified as under developed villages following the criteria of the Department of Community Development , 12 of them were chosen to be the sample villages of this study by drawing lots. Name and location of the studied villages were shown on figure III . The size of the selected villages varied from 80 to 120 house hold .

The poorly developed villages of Amphoe Ban Fang is chosen to be the study area of this study because :

1. The prevalence of anemia in rural villages especially in the poor area is higher than in Bangkok or in the other urban areas.
2. The population of Amphoe Ban Fang have the same culture, eating habits and belief as most of the population of the northeastern part of Thailand.
3. It is not too far from Khon Kaen University, so that it is possible to bring the specimens back to laboratory room within 6 hours .

The map of Khon-Kaen province and Amphur Banfang are shown at figure 2 and 3 .

FIGURE II : MAP OF KHON-KAEN PROVINCE

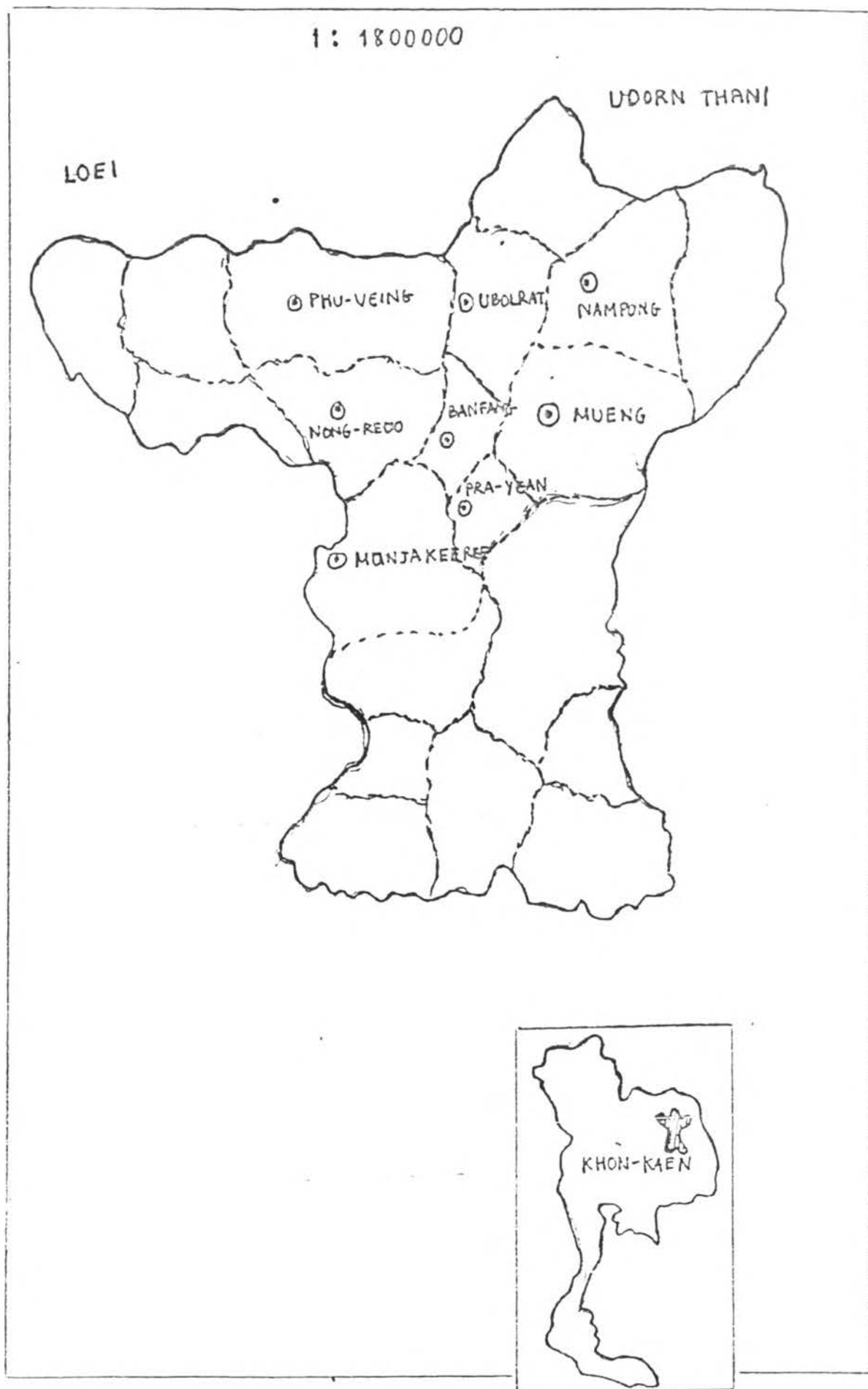
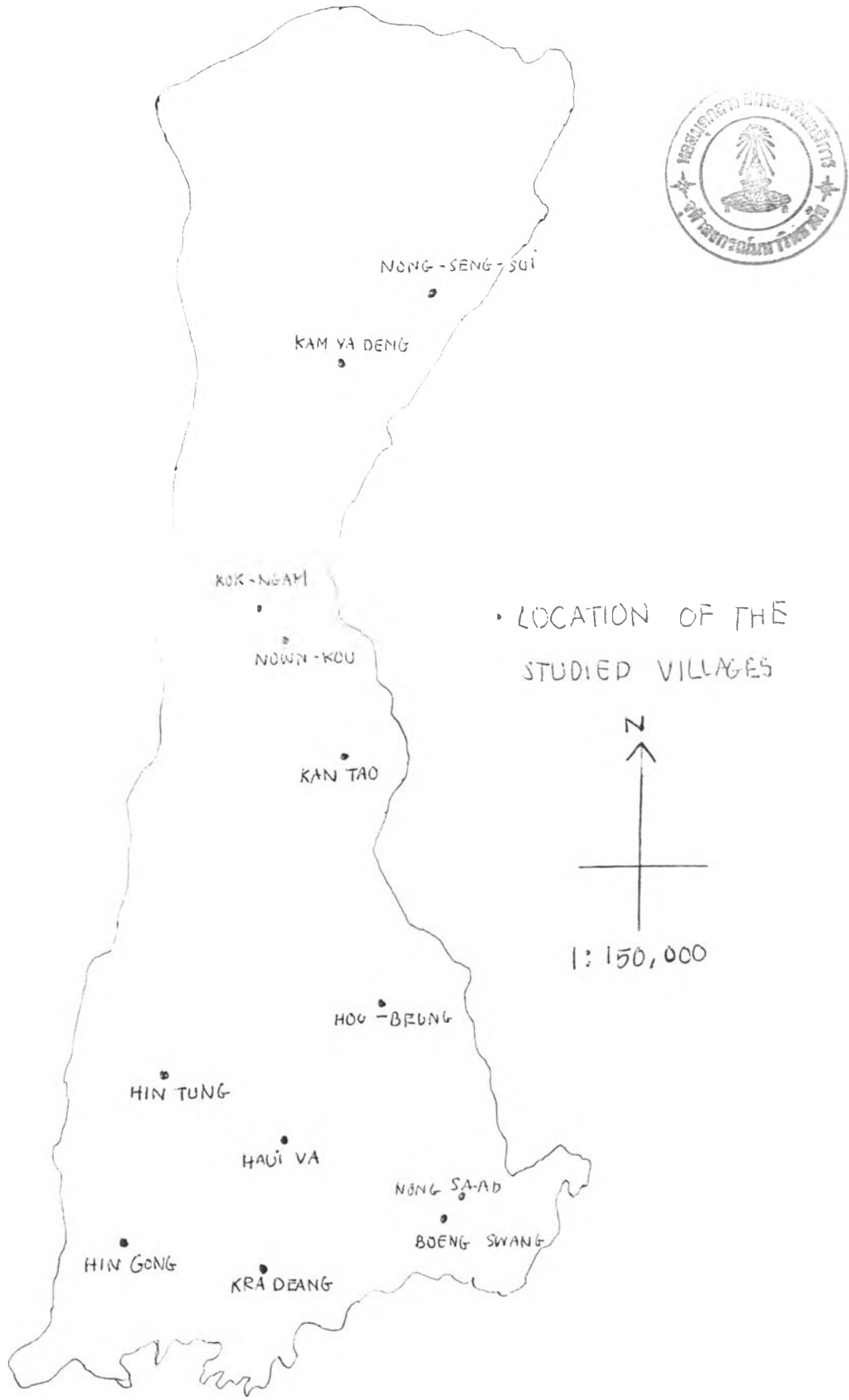


FIGURE III : MAP OF AMPHOE BAN-FANG



## SAMPLE SELECTION

Cluster sampling using villages as the sampling unit was used in this study. There are 25 villages in Amphoe Ban Fang which was classified by the Department of Community Development as the poorly developed villages following the criteria that use to classified the villages all over Thailand. The name of these villages was listed. Then 12 villages was chosen randomly by drawing lots.

The name of the women who lived in the chosen villages and born between 1948 and 1978 were listed by using the record of community health center. After that the name of pregnant, lactating woman and those who migrated to work and lived at the other place at the study time were excluded from the list by the officers of community health center and the health volunteers of the villages. All of the women who have the name in the list and did not meet any exclusion criteria and agree for blood extraction was used as the study subjects.

## MEASUREMENT

The variables which were measured in this study are :

1. hematocrit
2. serum ferritin
3. iron deficiency anemia
4. factors related to iron deficiency anemia



- Blood loss
  - menstrual history
  - number of offspring
  - age of youngest offspring
  - history of hematemesis , melena
  - history of aspirin intake
  - hook worm infestation
  - stool : occult blood
  
- Iron intake
  - type of diet
  - migration to big city

## 1. Hematocrit

Hematocrit determination was done by one technician immediately after blood samples were drawn from the subjects.

The method of measurement used was microcentrifuge technique. Blood sample was put into two heparinized capillary tube, then centrifuge in microhematocrit centrifuge machine at 2000 g. for 5 minutes, to obtain the ratio of packed cell volume and total blood volume . Each specimen was measured twice separately. The average value of the two was used as the hematocrit value.

We used only one microhematocrit centrifuge machine to avoid error that may cause by the difference of machines.

The subjects were determined as anemia when hematocrit was less than 36%,(Follow WHO criterion for anemia in female).

## 2. Serum ferritin

Serum ferritin was done by ELISA method using commercial kits (Enzymun-Test manufactures by Boehringer Mannheim GmbH. Company). The procedure was done by only one technician who knew nothing about the samples or results of other laboratory procedures done.

The reason for using serum ferritin and ELISA technique in measuring it was mentioned in the beginning under "Background and Rationale".

While measuring the blood sample, the laboratory kits was standardized by measuring 6 standard serum ,one control known serum and reagent blank within the same time . The standard curve was constructed by plotting the absorbance values obtained for each ferritin standard against th corresponding ferritin concentration . The ferritin concentration of the sample can then be read off the standard curve . The quality control of the test was checked by the control known serum . The value of the control lies within the confidence limit of assigned value , the within run CV is 3.9-4.1 % .

The stability of ferritin in serum is about 6 months at  $-20^{\circ}\text{C}$  .The standard and control serum have been calibrated against the WHO International Reference Standard 80/602 , from the National Institute for Biological Standards and Control (NIBSC). The analytical sensitivity of the test is  $< 2 \text{ ng/ml}$  .

The subjects were identified as iron deficient if serum ferritin less than  $15 \text{ ng/ml}$ .

The subjects were identified as iron deficiency cases when

Hematocrit  $< 36\%$

Serum ferritin  $< 15 \text{ ng/ml}$

### 3. Factors related to iron deficiency anemia

This part of data was obtained using the interview method. The data sheet, interview guideline and instrument for helping the subjects to recall drugs used were created, tested and improved. The instrument for recalling drugs used was list name of common NSAID such as Tamjai , Boad-hai , Bura, etc. and the sample of NSAID commonly sold at drug store .The interviewers were trained and tested before the survey started. All interviewers are local people who can understand and talk the northeastern dialect well. The data obtained by interview method include:

#### 3.1 Menstrual history: The menstrual history question include :

- number of days of menstruation per cycle.
- regularity of menstruation
- number of sanitary pads used / cycle of menstruation

#### 3.2 Number of offspring and age of the youngest offspring

The number of offspring will be asked because it's related to the amount of blood loss during delivery. Every time a woman gets pregnant, she losses the iron storage and when she gives birth, there is blood loss also. So, the number of offspring she has and the age of the youngest offspring have an effect on her iron status.

#### 3.3 History of hematemesis and history of hemorrhoid bleeding

The subjects will be asked about the history of hematemesis , melena and hemorrhoid bleeding because they have direct correlation with chronic blood loss, the duration and possibly amount of bleeding were asked also.

### 3.4 History of aspirin or NSAID taking

Aspirin intake may cause gastritis that leads to gastrointestinal bleeding ; some people in the rural area use aspirin or NSAID to relieve muscle pain after their work and finally get addicted to it.

The history of taking, dose, duration and indication for using these kinds of drugs were asked.

The subjects were classified as "no use" ,"occasional use" and "regular use" group as mentioned in the assumption . The " regular use " were considered as aspirin or NSAID abuse.

### 3.5 Type of diet

Since a detailed history about diet intake needs so much time, personnel and budget . The time to conduct the study was limited because it had to be finished within one year, therefore the details about diet intake by each subject was not studied.

From the previous study and from theory, we knew that most of the absorbable iron came from heme-iron. Therefore, the vegetarian has higher risk to have iron deficiency anemia. We knew that it took about 3 year to eliminate all of the iron storage in the body of the people who had normal iron storage if they did not receive iron intake, therefore, in this study we classified the vegetarian as a subject who did not eat any kind of protein from animal source including egg for at least 3 years.

The habit of eating clay which was an iron inhibitor was asked also because it could lead to iron deficiency anemia. Amount, frequency and duration of eating clay were asked.

Usually food taboo in this region applied only among the sick people, the pregnant or lactating women which were not the target population in this study , so it was not asked.

The subjects are classified as :

- Vegetarian or non vegetarian
- Clay eaters or non clay eaters.

### 3.6 Migration to the big city

Migrating to a big city may change the economic status and the food habits of the people. The subjects who moved to work in the big city for more than 3 months a year within 3 years were considered as the migrant group. The group that did not meet the criterion were considered as non-migrant group.

### 3.7 Stool Exam

The subjects stools were tested for parasitic infestation using modified Kato's thick smear technique. For testing of stool occult blood the Guaiac test (Hemoccult kit) was used.

Before the survey started, the technician was tested by examining blinded 30 samples twice. The intra observer variation was calculated.

		observation 1		
		+	-	
observation 2	+	10	2	Po = 0.9
	-	1	17	Pc = 0.53
				KAPPA = 0.79



The modified Kato-Katz thick smear technique ( Katz , et.al. , 1972 ) was chosen to be the stool examination method because it can be easily performed , possible to estimate the number of parasite eggs and sensitive for detecting light infestation . However , it was not suitable for detecting protozoa and *Opisthorchis viverrini* egg because of the small size of parasite . The shape of hookworm egg may change during the process of this method.

## DATA COLLECTION

Before the survey started, we asked for cooperation from Ban Fang community hospital and primary health center officers. Since they are the people who work very close to the village leader and health volunteer, therefore they can help a lot about managing data collection. The objectives of the study were explained to them. Then list of the sample names were created as the detail in sample selection.

The suitable time of survey was arranged. After that each primary health center officer went to the village, explained objectives of the study including the expected benefit to the subjects then made the appointment with them.

The subjects were asked to bring their specimen of feces with them on the appointment day. The specimens were brought to the university laboratory to be examined.

The tentative subjects were undergone history taking and physical examination to rule out the person who had any exclusion criteria.

The persons who met the eligibility criteria were interviewed by the interviewer about the factors associated with iron deficiency as previously mentioned.

Each subject was asked for permission of blood extraction. If they permit, 5 CC of blood was drawn from the antecubital vein.

Hematocrit was measured immediately by microcentrifuge technique. The remaining blood was brought to the university's laboratory as soon as possible. Then it was centrifuged at 2000 g. for 5 minutes. The plasma was harvested and kept at -22° C waiting for plasma ferritin measurement at the end of the survey schedule.

After all of the laboratory measurement finished, all of the data were filled up in the data sheet and put into the computer programme. Then data analysis were done.

## LIMITATION

Because of limitation in the budget, man power and feasibility, simple random technique can not be used for selecting the subjects in this study. We have to use the cluster sampling technique using a village as the cluster unit. To overcome the disadvantage of cluster sampling technique, the sample size calculated according to the formula of simple random technique is multiplied by design effect of 2.

The migration rate of the people in this area is very high especially in dry season. They moved to the town or big city to find a job. Because this year the weather was so bad, there was almost no rain in rainy season, so many people could not grow their crops. This made more people migrate and they moved earlier than usual. The head of the villages, officer of primary health care center and the village health volunteers had been helped a lot in listing the name of the possible subjects, encouraged them to stay until the survey finished, prepared time and place of data collection, collected feces specimens and encouraged the subjects to permit blood extraction. Because of their influence and good relationship to the people, only 11 persons from 640 selected subjects refused blood extraction. The successful rate was about 99.8%

The successful rate of collecting feces was lower than that of collecting blood sample. Since some subjects could not bring their feces with them on the collecting data day because they did not pass their bowel on that day. We did



not have enough time and money to return to the villages more than two times and in some villages the subjects moved to work in the other town within one or two days after the collecting data day. In the other hands we had to examine more than 150 specimens of non subjects cases and treated them as the incentive for the community. Among these 2.0% of them infested by hookworm and 6.6% stool occult blood positive.

The comparison between the group which we could examine their feces and could not was done. No statistical significance was found.

To measure the exact amount of iron intake of each subject was a very complicated work. It needs so many personnels, budgets and laboratory tests. Because of the limitation of time and budget in this study, therefore the measurement of amount of iron intake of each subject and iron content in her food were not measured. It is assumed that the iron food content may at least equal the data from the previous study by Nutacharat, et al. in 1973 (12.3 mg/day). However, the results of prevalence of iron deficiency anemia from this study is not as high as expected, thus it may not cost-effective to perform this kind of study in this area.

From this study we did not have any vegetarian, so we can not compare the effect of vegetarian or non vegetarian on prevalence of iron deficiency anemia.

## DATA ANALYSIS

The results were analyzed by computer using EpiInfo programme. The descriptive statistic was used to identify :

- prevalence of anemia
- prevalence of iron deficiency anemia
- prevalence of iron deficiency
- mean hematocrit with standard deviation
- mean serum ferritin with standard deviation
- prevalence of iron overload

The factors associated with iron deficiency anemia were analyzed according to the following dummy table, using chi-square , Fishers exact test or unpaired T-test.

	MEAN AGE (YEARS)	STATISTIC
IRON DEF. NO IRON DEF.		UNPAIRED T-TEST

NUMBER OF OFFSPRING	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
0		
1		
2		
3		
≥4		

AGE OF YOUNGEST OFFSPRING	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
< 2 YEARS		
> 2 YEARS		

MENSTRUAL HISTORY	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
REGULAR		
IRREGULAR		

	MEAN DURATION OF MENSTRUATION	STATISTIC
IRON DEF. NO IRON DEF.		UNPAIRED T-TEST

	MEAN SANITARY PAD USED / CYCLE	STATISTIC
IRON DEF. NO IRON DEF.		

DIETARY TYPE	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
VEGETARIAN NON-VEGETARIAN  CLAY EATER NON-CLAY EATER		

ANALGESIC USED	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
NOT USED OCCASIONAL USED REGULARY USED		

GI BLOOD LOSS	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
HX. OF GI BLEED NO HX.OF GI BLEED  HX. OF HEMORRHOID NO HX.OF HEMORRHOID		

BIRTH CONTROL	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
NO BIRTH CONTROL STERILIZATION IUD'S OTHERS		

MIGRATION	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
MIGRATE		
NOT MIGRATE		

STOOL EXAM	IRON DEF. ANEMIA	NO IRON DEF. ANEMIA
NO PARASITE		
HOOK WORM		
OTHER PARASITE		
OCCULT BLOOD+VE		
OCCULT BLOOD-VE		